



MINI REVIEW ARTICLE

Target genes utilized for drought tolerance enhancement in maize

Mukhammadjon K. Mirzakhmedov^{1*}, Lola K. Kamalova¹, Mirzakamol S. Ayubov¹, Kunduz T Normurodova²,
Khurshida A. Ubaydullaeva¹, Zabardast T. Buriev¹, Ibrokhim Y. Abdurakhmonov¹

¹Bioinformatics Laboratory, Center of Genomics and Bioinformatics, Uzbekistan Academy of Sciences of, University Street 2, Tashkent, 111215, Uzbekistan

²Department of Microbiology and Biotechnology, Biology faculty, National University of Uzbekistan, University Street 4, Tashkent, 100174, Uzbekistan

*Email: mirzakhmedov.m@gmail.com



OPEN ACCESS

ARTICLE HISTORY

Received: 31 March 2023

Accepted: 05 October 2023

Available online

Version 1.0 : 19 October 2023

Version 2.0 : 24 October 2023



Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care etc. See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

Copyright: © The Author(s). This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited (<https://creativecommons.org/licenses/by/4.0/>)

CITE THIS ARTICLE

Mirzakhmedov M K, Kamalova L K, Ayubov M S, Normurodova K T, Ubaydullaeva K A, Buriev Z T, Abdurakhmonov I Y. Target genes utilized for drought tolerance enhancement in maize. Plant Science Today. 2023; 10(sp2): 249-254. <https://doi.org/10.14719/pst.2561>

Abstract

Maize, which is consumed by millions of people worldwide as a staple food, is among the most commonly cultivated cereal crops. It is primarily used for human consumption in various forms, animal feed, and industrial applications. In many countries like Mexico, Africa, and South America, it is the main source of calories in their daily diet, making it crucial for food security. Many nations worldwide are more at risk of drought as global warming continues to accelerate. One of the major hurdles to food production in the twenty-first century and a serious threat to our present and future food security is a water crisis. Crop failure due to water scarcity can put millions of lives at risk. Along with traditional breeding, transgenic approaches are an essential tool in modern plant breeding. They allow the introduction of beneficial genes from other organisms or within the same organism to improve plant characteristics. This review focuses on specific genes that are stably expressed and tested for drought tolerance in maize. Several genes have been identified as potential targets for improving drought tolerance in maize. Although mechanisms of target genes overlap to some extent, we attempted to divide the selected research articles according to the mechanism of the targeted gene into categories and reviewed them.

Keywords

Drought stress; maize; overexpression; transgene

Introduction

Drought appears to be the most expensive disaster when compared to floods, wildfires, diseases, and infestations. Between 2005 and 2015, it caused 30 percent of agricultural losses, amounting to over USD 29 billion in developing countries. Drought has a more significant impact on the agriculture sector, bearing 83 percent of all the damage and losses caused by drought (1).

Drought stress poses a prevalent and severe environmental stressor that plants encounter, particularly in regions with arid and semi-arid climates. It occurs when soil moisture levels fall below the critical threshold required for plant growth and development. Consequently, the physiological, biochemical, and molecular alterations that occur in response to drought stress can adversely affect crop yield and productivity in plants. Drought stress can affect plant growth by influencing processes such as photosynthesis, water uptake and transport, nutrient absorption, and protein synthesis. Plants respond to drought stress by activating a range of adaptive mechanisms, including osmotic adjustment, antioxidant defense, and stomatal closure (2,3). Drought tolerance in crops refers to the ability of plants to survive extended periods of water scarcity, reducing yield losses

and maintaining productivity. Through advancements in genetic engineering, molecular biology techniques, and agronomic practices, researchers have identified and developed numerous mechanisms to improve drought tolerance in various crop species. These mechanisms include enhancing root growth and development, improving water use efficiency, and regulating hormone signaling pathways. This ongoing research is critical for ensuring food security and sustaining agricultural production in regions experiencing water stress and climate change.

The detrimental impact of water scarcity on crop yield can vary significantly, ranging from 30% to as high as 90%. The extent of this impact depends on the crop's growth stage and the severity and duration of water deficiency. In the case of maize, vulnerability to water scarcity is most pronounced during its vegetative, silking (flowering), and ear (grain filling) stages, where potential yield losses can reach up to 25%, 50%, and 21%, respectively (4).

In this review, we aim to provide a comprehensive overview of specific genes that hold promise in enhancing drought tolerance in maize. By categorizing and reviewing these genes based on their respective mechanisms, we aim to shed light on the diverse strategies employed to improve maize's resilience against drought.

Phytohormone Signaling Pathways

To make plants more drought-resistant, researchers are investigating phytohormone signaling pathways to enhance drought resistance in plants. Phytohormones like ABA, SA, JA, and auxin are key players in these pathways, regulating processes such as stomatal closure and root growth to help plants withstand drought stress. Exogenous application of phytohormones is being explored to boost drought tolerance. However, understanding how these hormones interact in response to drought remains complex (5). ABA is a hormone in plants that plays a crucial role in defending against various abiotic stresses and is a primary focus in developing drought tolerance (6) (Fig. 1).

Overexpressing the ZmPIS gene in maize, which plays a key role in phosphatidylinositol synthesis, triggered the activation of genes involved in both the ABA biosynthesis and phospholipid metabolism pathways. This, in turn, led to the development of transgenic maize lines with enhanced drought tolerance, especially before flowering (8).

ZmPP2C-A10 (protein phosphatase 2C gene) overexpression in Maize and Arabidopsis demonstrated that the gene negatively controls drought tolerance. Further evidence for the theory comes from a naturally occurring deletion of the endoplasmic reticulum stress response element in the 5'-UTR region of ZmPP2C-A10, which led to an increase in drought tolerance (9).

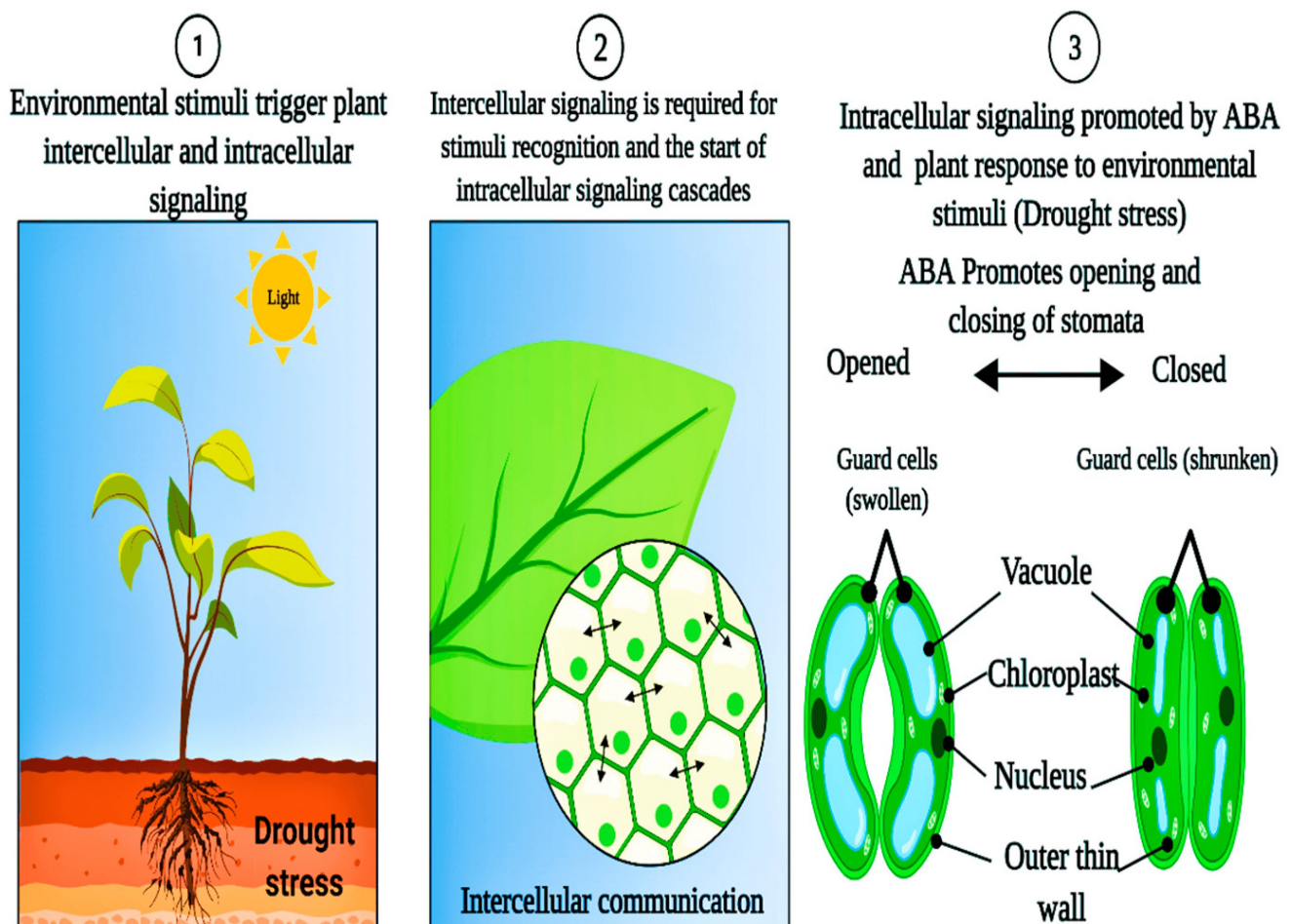


Fig. 1. When plants encounter biotic and abiotic stimuli, ABA is transported to guard cells, which subsequently initiates the closure of stomata in leaves. The Figure was re-used from (7) with the permission of MDPI

The 9-cis-epoxy carotenoid dioxygenase (NCED) and zeaxanthin epoxidase (ZEP) encoding key enzymes in ABA biosynthesis were downregulated by BMV-ZmbZIP33 (basic region/leucine zipper motif) mutants created by virus-induced gene silencing (VIGS) under circumstances of drought stress and rewatering but increased when ZmbZIP33 was overexpressed (10).

Although ethylene's role in the senescence of plants has been thoroughly investigated, less is known about its function during senescence brought on by water shortage (11). Depending on the plant species and the degree of drought stress, different ethylene responses can take place. Reduced ethylene sensitivity was achieved by overexpressing the ARGO genes, and transgenic lines under both drought stress and well-watered conditions produced better yields (12).

The AP2/ERF (APETALA2/ETHYLENE RESPONSIVE FACTOR) transcription factor family is essential for plant development and stress responses. Overexpression of ZmEREBP60 (ethylene-responsive element binding proteins), one of its family members in maize generated drought stress tolerant lines by reducing H₂O₂ accumulation and malondialdehyde content (13).

Increasing Photosynthesis Rate

Photosynthesis is the primary target affected by water stress. It reduces photosynthetic rates by limiting the availability of carbon dioxide (CO₂) due to stomatal closure. Water stress also triggers the production of harmful reactive oxygen species (ROS), such as hydrogen peroxide (H₂O₂), which negatively impacts photosynthesis (14). Maintaining the rate of photosynthesis under water shortage is considered a valuable trait. The NPK1 gene, a tobacco mitogen-activated protein kinase kinase kinase (MAPKKK), plays a crucial role in improving drought tolerance in maize. When NPK1 is expressed constitutively in maize, it enhances the plant's ability to withstand drought conditions. This enhancement is primarily attributed to NPK1's activation of an oxidative stress signaling pathway. Under drought stress, transgenic maize plants with NPK1 expression maintain higher photosynthesis rates compared to non-transgenic plants. This protection of the photosynthesis machinery from dehydration damage contributes to improved drought tolerance. Additionally, NPK1 expression leads to delayed maturation and increased leaf numbers in maize plants under drought conditions. Importantly, NPK1-expressing maize plants produce kernels with weights similar to those under well-watered conditions, whereas non-transgenic plants exhibit a significant reduction in kernel weights when exposed to drought. This suggests that NPK1 expression enhances the yield potential of maize under drought stress by protecting photosynthesis and mitigating the adverse effects of water deficit (15).

Vacuolar-type H⁺ pyrophosphatase (ZmVPP1) a gene in maize, has a significant role in enhancing drought tolerance. The study revealed that an insertion of 366 base pairs in the promoter region of ZmVPP1, containing three MYB (myeloblastosis viral oncogene homolog) cis-elements, leads to drought-inducible expression of this

gene in drought-tolerant maize varieties. When maize plants are genetically modified to have increased ZmVPP1 expression, they display improved drought tolerance and authors suggested it is likely because of maintaining photosynthetic efficiency and root development under drought stress (16).

Researchers discovered that an 82-bp MITE insertion in its promoter region of the ZmNAC111 gene is linked to drought resistance. This insertion represses ZmNAC111 expression through DNA and histone methylation via the RdDM pathway. The MITE insertion appears to have occurred after maize domestication and is more common in temperate maize germplasm (17). In field trials, maize lines co-expressing ZmVPP1 and ZmNAC111 (NAM, ATAF1,2, and CUC (NAC)-type transcription factor) genes demonstrated elevated photosynthesis rates, increased antioxidant enzyme activity, and greater root-to-shoot ratios during the seedling stage, surpassing those observed in the wild-type counterparts. Additionally, these modified lines exhibited shortened anthesis-silking intervals and yielded more grains when confronted with water scarcity (18).

The Nuclear Factor-Y (NF-Y) family of transcription factors is a complex regulatory system in plants that plays a pivotal role in growth, development, and stress responses. This complex is made up of NF-YA, NF-YB, and NF-YC subunits, forming heterotrimeric complexes. NF-Ys regulate gene expression by binding to specific DNA sequences and interacting with other transcriptional regulators (19). Overexpression of ZmNF-YB16, a member of the NF-YB superfamily, in maize plants during both vegetative and reproductive stages increases resistance to dehydration and drought stress by maintaining increased photosynthesis rate, bolstering antioxidant enzyme activity, reinforcing cellular stress responses and improves maize grain yield under normal and drought stress conditions. ZmNF-YB16 was found to regulate the expression of several genes involved in photosynthesis, cellular antioxidant capacity, and the endoplasmic reticulum stress response during drought stress in maize. (20).

TPP (TREHALOSE PHOSPHATE PHOSPHATASE) is a key player in maize's response to drought stress, particularly during the critical flowering phase. It regulates sucrose metabolism in developing ear spikelets, enhancing kernel set and ultimately increasing maize crop yield (21). Transgenic maize that expresses OsTPP1 under MADS6 (MCM1, AG, DEF, SRF) promoter which is functional throughout the flowering phase generates more yield compared to wild type regardless of with or without drought conditions. The MADS6 promoter activated mainly in vasculature tissues, in line with a significant reduction of trehalose 6-phosphate (T6P) levels, altering gene expression toward secondary metabolism, enhancing resource allocation, and boosting crop yield under varying conditions, including drought during flowering, while maintaining higher photosynthetic rates (22).

Water Use Efficiency

The concept of water use efficiency (WUE) relates to the measure of how much biomass or yield is produced by a plant per unit of water consumed. In drought conditions, WUE becomes an important trait for plants as they need to conserve water while still producing adequate yield (23). Under mild drought circumstances, phosphoenolpyruvate carboxylase (C(4)-PEPC) overexpressing maize lines showed an increase of up to 30% in intrinsic WUE as well as an increase of 20% in dry weight. Underexpressing the relevant proteins, resulted in the opposite effect (24).

Maize plants that were genetically modified to overexpress phosphoinositide phospholipase C1 (ZmPLC1) exhibited improved characteristics such as higher relative water content (RWC), better osmotic adjustment, increased rates of photosynthesis, lower percentage of ion leakage, and reduced lipid membrane peroxidation when compared to non-genetically modified plants (25).

Transgenic maize with higher ZmNAC111 expression exhibited improved WUE and increased seedling drought tolerance (26).

Ectopic expression of SbER2-1 (ERECTA) in both Arabidopsis and maize plants led to an improvement in drought tolerance, specifically in terms of (WUE), as demonstrated by increased net photosynthetic rates in maize under drought conditions (27).

Raffinose synthase (RAFS) is a crucial enzyme in plants that produces raffinose, a trisaccharide with diverse functions. Raffinose helps plants withstand abiotic stress, maintains photosynthesis, and stabilizes cell membranes. It also supports beneficial bacteria (28). ZmRAFS overexpression in maize improved plant resistance to drought stress by raising levels of RAFS protein, and raffinose content and reduced leaf water loss. Under normal watering conditions, the ZmRAFS transgenic plants had similar biomass to non-transgenic plants. However, under drought stress conditions, the transgenic plants had significantly higher biomass than the non-transgenic control plants. Although there were variations in water loss among different maize lines, there were no differences in stomatal density or aperture. The research suggests that overexpressing the ZmRAFS gene in maize, but not in Arabidopsis, increased raffinose levels in leaves, leading to improved water retention and increased drought resistance without affecting growth significantly (29).

Stomatal aperture

Stomatal closure is a vital initial response of plants to drought stress, closely linked to soil moisture levels and controlled by chemical signals like ABA produced in dehydrating roots. Various factors, including xylem ABA content, leaf-to-air vapor pressure deficit, sap pH, plant nutrition, hydraulic conductance, and more, influence stomatal regulation. While stomatal closure reduces CO₂ assimilation and net photosynthesis, it conserves water and aids in survival. Stomatal size and density can change under drought stress, with species-specific responses (30). The LOS5 gene, also known as ABA-deficient 5 (ABA3),

plays a pivotal role in the response of Arabidopsis plants to low temperature and osmotic stress. LOS5 acts as a key player in abscisic acid (ABA) biosynthesis, a hormone crucial for stress adaptation, by catalyzing the generation of a cofactor required for the final step of ABA production (31). Overexpression of the Arabidopsis molybdenum cofactor sulfurase gene (LOS5) in maize resulted in increased expression of ZmAo (aldehyde oxidase) and activity of AO, leading to the accumulation of ABA and enhanced drought resistance. The transgenic maize plants that overexpressed the Arabidopsis LOS5 gene exhibited reduced stomatal aperture, resulting in reduced water loss and better preservation of relative water content (RWC) and leaf water potential compared to wild-type plants under drought conditions. Additionally, the transgenic maize plants showed lower levels of leaf wilting, electrolyte leakage, malondialdehyde (MDA), and H₂O₂ content, increased activity of antioxidant enzymes, and higher levels of proline compared to the wild-type plants under drought stress (32).

ZmMEK1 (MAP kinase kinase) regulates stomatal closure and is inhibited by ZmPP48 through dephosphorylation. Active ZmMEK1 enhances ZmSIMK1 activity, which directly phosphorylates ZmSLAC1, promoting stomatal closure. This process is triggered when ZmPP84 transcription decreases under drought stress, releasing ZmMEK1 inhibition and activating ZmSIMK1 for stomatal closure. By dephosphorylating ZmMEK1 (MAP kinase kinase), ZmPP84, a negative regulator of the drought stress response, prevents stomatal closing (33).

Improving Root System

With the absorption of water and nutrients from the soil, plant roots actively participate in the growth of plants. To improve plant productivity in drought conditions, it is believed that certain root characteristics, such as fine root diameter, specific root length, specific root area, root angle, and root length density, can be beneficial. (34). Overexpression of ZmPIN1a, which is a component of the auxin efflux carrier, in maize resulted in the development of a more extensive root system with longer primary roots and denser lateral roots. This was due to an increase in the number of lateral roots and a decrease in their elongation. At the same time ear height, internode length, and plant height decreased in transgenic lines. The altered root structure resulting from the overexpression of ZmPIN1a in maize plants led to improved productivity under high-density cultivation, and enhanced plant resilience to drought, lodging, and low-phosphate conditions. ZmPIN1a overexpression enhanced IAA transport from shoot to root, as shown by measurements of IAA concentration, transport capacity, and exogenous IAA application. The plant was able to provide the roots with additional carbohydrates, which promoted root growth and strengthened the plant's ability to withstand environmental stress due to the rise in auxin in the root (35).

The transgenic maize plants overexpressing ZmPTF1 showed improved tolerance to drought stress as a

result of their enhanced root structure, increased levels of abscisic acid (ABA), and activation of stress responses mediated by CBF4, ATAF2, and NAC30 whereas the mutants with reduced expression of ZmPTF1 showed the opposite results (36).

In transgenic *Arabidopsis* and maize, upregulating the expression of ZmTIP1 (TIP GROWTH DEFECTIVE1) resulted in longer root hairs and enhanced plant tolerance to water deprivation. On the other hand, ZmTIP1 transposon-insertional mutants exhibited the opposite phenotype (37).

Conclusion

Drought stress is a widespread issue that affects plant growth, quality, and energy production, transcending geographical boundaries without restraint. Traditional methods and natural plant adaptations might not be enough to tackle this challenge. Genetic engineering is a promising technique, that enables scientists to modify the genes of plants, enhancing their ability to withstand drought stress. As evidenced by our observations, research endeavors aimed at enhancing drought tolerance in maize through transgenic approaches have predominantly centered around the overexpression of specific genes. Additionally, it has been demonstrated that the ectopic expression of certain genes has yielded highly promising results. Consequently, it would be highly beneficial to witness an increase in the expression of homologous genes within the maize genome itself. Furthermore, the utilization of genome editing tools to silence or entirely knockout genes that act as negative regulators of drought response genes holds the potential to reveal new opportunities in our pursuit of mitigating drought stress.

Acknowledgements

The work was done according to project #A-FA-2021-466, funded by The Ministry of Innovative Development of the Republic of Uzbekistan. We acknowledge the Center of Genomics and Bioinformatics research team for helping to collect information to write the manuscript.

Authors' contributions

MM and MA – wrote the manuscript; LK, QN, and KU collected and analyzed world literature; MA–critically read and edited the manuscript, and drafted subsections; ZB and IA edited and approved the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

References

1. FAO. 2018. The impact of disasters and crises on agriculture and food security: 2017. Rome. <http://www.fao.org/3/I8656EN/i8656en.pdf>

2. Chaves MM. Mechanisms underlying plant resilience to water deficits: Prospects for water-saving agriculture. *Journal of Experimental Botany*. 2004;55(407):2365–84. <https://doi.org/10.1093/jxb/erh269>
3. Farooq M, Wahid A, Kobayashi N, Fujita D, Basra SM. Plant drought stress: Effects, mechanisms and management. *Agronomy for Sustainable Development*. 2009;29(1):185–212. <https://doi.org/10.1051/agro:2008021>
4. Sah RP, Chakraborty M, Prasad K, Pandit M, Tudu VK, Chakravarty MK, et al. Impact of water deficit stress in maize: Phenology and yield components. *Scientific Reports*. 2020;10(1). <https://doi.org/10.1038/s41598-020-59689-7>.
5. Iqbal S, Wang X, Mubeen I, Kamran M, Kanwal I, Díaz GA, et al. Phytohormones trigger drought tolerance in crop plants: Outlook and future perspectives. *Frontiers in Plant Science*. 2022; 12. doi:10.3389/fpls.2021.799318
6. Daszkowska-Golec A, Hossain MA, Wani SH, Bhattacharjee S, Burritt DJ, Tran LS. *Drought Stress Tolerance in Plants, Vol 2 Volume 2*. Springer; Berlin/Heidelberg, Germany: 2016. pp. 123–151.
7. Wahab, A., Abdi, G., Saleem, M. H., Ali, B., Ullah, S., Shah, W., Mumtaz, S., Yasin, G., Muresan, C. C., & Marc, R. A. (). Plants physio-biochemical and Phyto-hormonal responses to alleviate the adverse effects of Drought stress: A comprehensive review. *Plants*. 2022; 11(13), 1620. <https://doi.org/10.3390/plants11131620>
8. Liu X, Zhai S, Zhao Y, Sun B, Liu C, Yang A, et al. Overexpression of the phosphatidylinositol synthase gene (ZmPIS) conferring drought stress tolerance by altering membrane lipid composition and increasing ABA synthesis in maize. *Plant Cell Environ*. 2013;36(5):1037-55. <https://doi.org/10.1111/pce.12040>
9. Xiang Y, Sun X, Gao S, Qin F, Dai M. Deletion of an Endoplasmic Reticulum Stress Response Element in a ZmPP2C-A Gene Facilitates Drought Tolerance of Maize Seedlings. *Mol Plant*. 2017;10(3):456-469. <https://doi.org/10.1016/j.molp.2016.10.003>
10. Cao L, Lu X, Wang G, Zhang Q, Zhang X, Fan Z, et al. Maize ZmZIP33 Is Involved in Drought Resistance and Recovery Ability Through an Abscisic acid-dependent Signaling Pathway. *Front Plant Sci*. 2021;12:629903. <https://doi.org/10.3389/fpls.2021.629903>
11. Arraes FB, Beneventi MA, Lisei de Sa ME, Paixao JF, Albuquerque EV, Marin SR, et al. Implications of ethylene biosynthesis and signaling in soybean drought stress tolerance. *BMC Plant Biology*. 2015;15(1). <https://doi.org/10.1186/s12870-015-0597-z>
12. Shi J, Habben JE, Archibald RL, Drummond BJ, Chamberlin MA, Williams RW, et al. Overexpression of ARGOS Genes Modifies Plant Sensitivity to Ethylene, Leading to Improved Drought Tolerance in Both *Arabidopsis* and Maize. *Plant Physiol*. 2015;169(1):266-82. <https://doi.org/10.1104/pp.15.00780>
13. Zhu Y, Liu Y, Zhou K, Tian C, Aslam M, Zhang B, et al. Overexpression of ZmEREBP60 enhances drought tolerance in maize. *J Plant Physiol*. 2022;275:153763. <https://doi.org/10.1016/j.jplph.2022.153763>
14. Osakabe Y, Osakabe K, Shinozaki K, Tran LSP. Response of plants to water stress. *Frontiers in Plant Science* 2014; 5. <https://doi.org/10.3389/fpls.2014.00086>
15. Shou H, Bordallo P, Wang K. Expression of the Nicotiana protein kinase (NPK1) enhanced drought tolerance in transgenic maize. *J Exp Bot*. 2004;55(399):1013-19. <https://doi.org/10.1093/jxb/erh129>
16. Wang X, Wang H, Liu S, Ferjani A, Li J, Yan J, et al. Genetic variation in ZmVPP1 contributes to drought tolerance in maize

- seedlings. *Nat Genet.* 2016;48(10):1233-41. <https://doi.org/10.1038/ng.3636>
17. Mao H, Wang H, Liu S, Li Z, Yang X, Yan J, et al. A transposable element in a NAC gene is associated with drought tolerance in maize seedlings. *Nature Communications.* 2015;6(1). <https://doi.org/10.1038/ncomms9326>
 18. Liu S, Liu X, Zhang X, Chang S, Ma C, Qin F. Co-Expression of ZmVPP1 with ZmNAC111 Confers Robust Drought Resistance in Maize. *Genes (Basel).* 2022;14(1):8. <https://doi.org/10.3390/genes14010008>
 19. Kavi Kishor PB, Ganie SA, Wani SH, Guddimali R, Karumanchi AR, Edupuganti S, et al. Nuclear factor (NF-y): Developmental and stress-responsive roles in the plant lineage. *Journal of Plant Growth Regulation.* 2022; 42(5), 2711–2735. <https://doi.org/10.1007/s00344-022-10739-6>
 20. Wang B, Li Z, Ran Q, Li P, Peng Z, Zhang J. ZmNF-YB16 Overexpression Improves Drought Resistance and Yield by Enhancing Photosynthesis and the Antioxidant Capacity of Maize Plants. *Front Plant Sci.* 2018;9:709. <https://doi.org/10.3389/fpls.2018.00709>
 21. Nuccio ML, Wu J, Mowers R, Zhou HP, Meghji M, Primavesi LF, et al. Expression of trehalose-6-phosphate phosphatase in maize ears improves yield in well-watered and drought conditions. *Nature Biotechnology,* 2015; 33(8), 862–869. <https://doi.org/10.1038/nbt.3277>
 22. Oszvald M, Primavesi LF, Griffiths CA, Cohn J, Basu SS, Nuccio ML et al. Trehalose 6-Phosphate Regulates Photosynthesis and Assimilate Partitioning in Reproductive Tissue. *Plant Physiol.* 2018;176(4):2623-2638. <https://doi.org/10.1104/pp.17.01673>
 23. Hatfield JL, Dold C. Water-use efficiency: Advances and challenges in a changing climate. *Frontiers in Plant Science.* 2019;10. <https://doi.org/10.3389/fpls.2019.00103>
 24. Jeanneau M, Gerentes D, Foueillassar X, Zivy M, Vidal J, Toppan A, et al. Improvement of drought tolerance in maize: towards the functional validation of the Zm-Asr1 gene and increase of water use efficiency by over-expressing C4-PEPC. *Biochimie.* 2002;84(11):1127-35. [https://doi.org/10.1016/s0300-9084\(02\)00024-x](https://doi.org/10.1016/s0300-9084(02)00024-x)
 25. Wang CR, Yang AF, Yue GD, Gao Q, Yin HY, Zhang JR. Enhanced expression of phospholipase C 1 (ZmPLC1) improves drought tolerance in transgenic maize. *Planta.* 2008;227(5):1127-40. <https://doi.org/10.1007/s00425-007-0686-9>
 26. Mao H, Wang H, Liu S, Li Z, Yang X, Yan J, et al. A transposable element in a NAC gene is associated with drought tolerance in maize seedlings. *Nat Commun.* 2015;6:8326. <https://doi.org/10.1038/ncomms9326>
 27. Li H, Han X, Liu X, Zhou M, Ren W, Zhao B, et al.. A leucine-rich repeat-receptor-like kinase gene SbER2-1 from sorghum (*Sorghum bicolor* L.) confers drought tolerance in maize. *BMC Genomics.* 2019 Oct 15;20(1):737. <https://doi.org/10.1186/s12864-019-6143-x>
 28. Xu J, You X, Leng Y, Li Y, Lu Z, Huang Y, et al. Identification and alternative splicing profile of the raffinose synthase gene in grass species. *International Journal of Molecular Sciences.* 2023; 24(13), 11120. <https://doi.org/10.3390/ijms241311120>
 29. Liu Y, Li T, Zhang C, Zhang W, Deng N, Dirk LMA, et al. Raffinose positively regulates maize drought tolerance by reducing leaf transpiration. *Plant J.* 2023 Jan 26. <https://doi.org/10.1111/tbj.16116>
 30. Pirasteh-Anosheh H, Saed-Moucheshi A, Pakniyat H, Pesarakli M. Stomatal responses to drought stress. *Water Stress and Crop Plants.* 2016; 24–40. doi:10.1002/9781119054450.ch3
 31. Xiong L, Ishitani M, Lee H, Zhu JK. The arabidopsis *Los5/ABA3* locus encodes a molybdenum cofactor sulfuryase and modulates cold stress- and osmotic stress-responsive gene expression. *The Plant Cell.* 2001; 13(9), 2063–2083. doi:10.1105/tpc.010101
 32. Lu Y, Li Y, Zhang J, Xiao Y, Yue Y, Duan L, et al.. Overexpression of *Arabidopsis* molybdenum cofactor sulfuryase gene confers drought tolerance in maize (*Zea mays* L.). *PLoS One.* 2013;8 (1):e52126. <https://doi.org/10.1371/journal.pone.0052126>
 33. Guo Y, Shi Y, Wang Y, Liu F, Li Z, Qi J, et al. The clade F PP2C phosphatase ZmPP84 negatively regulates drought tolerance by repressing stomatal closure in maize. *New Phytol.* 2023 Mar;237 (5):1728-1744. <https://doi.org/10.1111/nph.18647>
 34. Wasaya A, Zhang X, Fang Q, Yan Z. Root phenotyping for drought tolerance: A Review. *Agronomy.* 2018;8(11):241. <https://doi.org/10.3390/agronomy8110241>
 35. Li Z, Zhang X, Zhao Y, Li Y, Zhang G, Peng Z, Zhang J. Enhancing auxin accumulation in maize root tips improves root growth and dwarfs plant height. *Plant Biotechnol J.* 2018 Jan;16(1):86-99. <https://doi.org/10.1111/pbi.12751>
 36. Li Z, Liu C, Zhang Y, Wang B, Ran Q, Zhang J. The bHLH family member ZmPTF1 regulates drought tolerance in maize by promoting root development and abscisic acid synthesis. *J Exp Bot.* 2019 Oct 15;70(19):5471-5486. <https://doi.org/10.1093/jxb/erz307>
 37. Zhang X, Mi Y, Mao H, Liu S, Chen L, Qin F. Genetic variation in ZmTIP1 contributes to root hair elongation and drought tolerance in maize. *Plant Biotechnol J.* 2020 May;18(5):1271-1283. <https://doi.org/10.1111/pbi.13290>